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Nickel Deficiency Diminishes Sperm Quantity and Movement in Rats**,†

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ABSTRACT

Early studies on nickel essentiality with rats and goats indicated that nickel deprivation impaired reproductive performance. Nickel also has been found to influence cyclic nucleotide gated channels (CNG); these types of channels are important in sperm physiology. Thus, two experiments were conducted to test the hypothesis that nickel deficiency affects sperm physiology in a manner consistent with nickel having an essential function related to CNG channel functions. The experiments were factorially arranged with four treatment groups of eight weanling rats in each. In experiment 1, the treatments were supplemental dietary nickel of 0 and 1 mg/kg and N^{ω}-nitro-L-arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor) added to the drinking water (50 mg/100 mL) the last 3 wk of an 8-wk experiment. In experiment 2, the treatments were supplemental

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dietary nickel at 0 and 1 mg/kg and supplemental dietary sodium chloride (NaCl) at 0 and 80 g/kg. The NaCl and L-NAME variables were included to act as stressors affecting CNG channel activity. The basal diet contained per kilogram about 27 μg of nickel and 1 g of sodium. After 8 wk in experiment 1 and 16 wk in experiment 2, urine while fasting and testes and epididymis in both experiments, and seminal vesicles and prostates in experiment 2 were harvested for analysis. Nickel deprivation significantly decreased spermatozoa motility and density in the epididymides, epididymal transit time of spermatozoa, and testes sperm production rate. Nickel deficiency also significantly decreased the weights of the seminal vesicles and prostate glands. Excessive NaCl had no effect on sperm physiology; however, it decreased prostate gland weights. The findings support the hypothesis that nickel has an essential function that possibly could affect reproductive performance in higher animals, perhaps through affecting a CNG channel function.

Index Entries: Nickel; sodium chloride; L-NAME; spermatozoa; reproduction; trace elements.

INTRODUCTION

Early studies of the nutritional essentiality of nickel indicated that it is beneficial for reproductive performance of experimental animals. Nielsen et al. *(1)* reported that nickel deprivation during reproduction in rats increased perinatal mortality with a malnourished appearance (underweight and unthrifty) in pups before death. This study, however, did not determine breeding performance. Anke and co-workers *(2)* found that nickel deprivation of breeding goats significantly decreased the success of first insemination and conception rate and increased the number of breeding attempts to achieve pregnancy and the abortion rate. Follow-up studies to determine the mechanism through which nickel influenced reproductive performance apparently have not been done.

Another finding regarding nickel that has not received much attention is that it might be involved in cyclic nucleotide-gated (CNG) channel functions. Nickel potentiates cyclic guanosine monophosphate (cGMP)-gated cation channels in the rod outer segments of retinae, and it desensitizes CNG channels of olfactory receptor cells *(3–5)*. CNG channels are located in a number of organs, including the central nervous, urogenital, and reproductive systems *(6–12)*. In the reproductive system, CNG channels are found in spermatozoa.

The CNG channels also have an important role in kidney function and, thus, sodium metabolism. In the kidney, CNG-gated channels are located in the cortical collecting ducts and inner medullary collecting ducts of kidney; these cation channels are inhibited (closed) by the binding of cGMP. The closure of CNG cation channels decreases sodium reabsorption in the inner medullary collecting duct and results in natriuresis

(13,14). Chronic inhibition of nitric oxide (NO) synthase (e.g., feeding N^{ω}-nitro-L-arginine methyl ester [L-NAME]) in rats induces hypertension, decreased natriuresis, and renal damage *(13,15).* High-salt (NaCl) diets increase the urinary excretion of sodium and cGMP *(15).* Thus, feeding L-NAME and high-NaCl diets can be considered stressors of systems in which CNG channels are important.

If nickel is involved or needed for CNG channel function and/or for various guanylate cyclases upstream of CNG channels, nickel deficiency could affect a number of physiological functions, including vision, taste, olfaction, sodium metabolism, blood pressure control, cardiac function, and sperm physiology.

The objective of the described study was to ascertain whether nickel deficiency affects sperm physiology in such a way that it supports the hypothesis that nickel is involved in CNG channel functions. In an effort to enhance the effect or need for nickel if it is involved in a function related to CNG cation channels, L-NAME administration and high dietary NaCl were used as treatment variables.

MATERIALS AND METHODS

Experimental Design

Two factorially arranged experiments using weanling male Sprague–Dawley rats (Charles River/SASCO, Wilmington, MA) weighing 23–33 g were performed. In experiment 1, 32 rats were assigned to 4 weight-matched groups of 8, with 2 groups being fed the basal diet low in nickel and the other 2 groups fed the basal diet supplemented with 1 mg Ni/kg. At 5 wk, 50 mg L-NAME/100 mL were added to the drinking water of one each of the two dietary nickel-treatment groups. In experiment 2, the treatment variables of four weight-matched groups of 8 were supplemental dietary NaCl at 0 or 80 g/kg and supplemental dietary nickel at 0 or 1 mg/kg. The rats in both experiments were housed individually in plastic cages in laminar airflow racks. The racks were located in a room maintained at 23°C and 47% relative humidity with a 12-h light (7 PM to 7 AM; because of behavioral testing) and 12-h dark cycle. The rats had free access to food and deionized drinking water (Super Q, Millipore).

The composition of the basal diet is shown in Table 1. Nickel (as a mix of 0.405 g $NiCl_2 \cdot 6H_2O$ and 99.595g sucrose) and NaCl supplements replaced sucrose in the basal diet. One gram of basal diet contained about 27 ng of nickel and 1 mg of sodium as determined by atomic absorption spectrometry *(16)* and inductively coupled atomic emission spectrometry *(17),* respectively. Standard reference material (National Institute of Standards and Technology, Gaithersburg, MD) #1515 apple leaves was used for quality control purposes in the diet analyses.

Table 1
Basal Diet

Ingredient	g/kg diet
Vitamin-free casein[a]	150.00
Corn starch	150.00
Sucrose	526.50
Alphacel	50.00
Corn Oil	80.00
Vitamin mixture[b]	5.00
Mineral mixture[c]	35.00
L-Cystine	3.00
Choline chloride	0.50

[a] Sigma Chemical Co, St. Louis, MO.

[b] Vitamin mixture provided 0.75 mg vitamin K_1, 30 mg nicotinic acid, 0.2 mg biotin, 6 mg thiamin hydrochloride, 16 mg calcium pantothenate, 25 µg vitamin B_{12}, 18 mg *dl*-α-tocopherol acetate, 6 mg riboflavin, 1 mg folic acid, 7.5 mg pyridoxine hydrochloride, 2.20 mg retinyl palmitate, and 25 µg vitamin D_3 per kilogram of diet.

[c] Mineral mixture provided 12.5 g $CaCO_3$, 2.0 g KCl, 2.6 g NaCl, 8.8 g KH_2PO_4, 0.8 g MgO, 2.48 g ferric sulfate mixture, 10 mg ZnO, 40 mg $MnSO_4 \cdot 4H_2O$, 20 mg $CuSO_4 \cdot 5H_2O$, 0.5 mg KI, 0.6 mg Na_2SeO_3, 0.27 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 2 mg $Cr(C_2H_3O_2)_3 \cdot H_2O$, 6 mg NaF, 6 mg H_3BO_3, 0.5 mg NH_4VO_3, 2 mg $Na_2HAsO_4 \cdot 7H_2O$, and 102 mg $NaSiO_3 \cdot 9H_2O$ per kilogram of diet. The ferric sulfate mixture provided 35 mg iron per kilogram of diet.

Procedures

In experiment 1, 12 d after the L-NAME treatment started, some rats became extremely hypertensive, anorexic, and morbid; these rats, which were not included in the experimental analyses, were killed or died. The premature and unexpected loss of rats resulted in the earlier than planned termination of experiment 1; termination was 3 wk after the L-NAME treatment began and 8 wk after the experiment was initiated. In experiment 2, the rats were on the dietary regimen for 16 wk. In both experiments, before termination the rats were placed in metabolic cages with free access to drinking water and no access to diet for 16 h during which time urine was collected in plastic tubes kept on ice. Then, the rats were anesthetized with ether, and blood was collected with a heparinized syringe. Both testes were excised, weighed, frozen in liquid nitrogen, and stored at –70°C. Both epididymides were excised and weighed. The entire right epididymis was used for the determination of sperm number and motility. In

experiment 2, seminal vesicles were excised and incised to remove seminal fluid, weighed, and then stored in neutral formalin.

This study was approved by the Animal Care Committee of the Grand Forks Human Nutrition Research Center, and the lawfully acquired animals were maintained in accordance with the NIH guidelines for the care and use of laboratory animals.

Sperm Counts, Sperm Motility, Daily Sperm Production, and Epididymal Transit Time

Spermatozoa in the right epididymides were counted by a modified method of Reeves and Rossow *(18).* The epididymis was finely minced with anatomical scissors in 5 mL of physiological saline, placed on a rocker for 10 min, then allowed to sit at room temperature for 2 min. An aliquot of the supernatant fluid was diluted with a solution containing 5 g sodium bicarbonate and 1 mL formalin per 100 mL of water before total sperm number was determined by using a hemacytometer. Another aliquot of the supernatant fluid was diluted in physiological saline; in experiment 2, an aliquot was also diluted in Medium 199 (Sigma Chemical Co., St. Louis, MO). The number of motile and immotile sperm in these aliquots were counted by using a hemacytometer. Motility was defined as a sperm that showed any movement in the flagellum during a 30-s observation period. Sperm motility was expressed as a percentage of motile sperm in total sperm. Daily sperm production in testes and epididymal transit time of sperm were determined on the right testis in experiment 2 by the method of Robb et al. *(19).* Briefly, the right testis was frozen in liquid nitrogen and stored at –70°C. After thawing at room temperature, the tunica albuginea was removed and the contents were cut into pieces with anatomical scissors. The contents were put into a semimicro Waring blender with 25 mL of 0.15 *M* NaCl/0.05% (w/v) Triton X-100/0.25 *M* merthiolate and homogenized for 1 min. The homogenate was stored at 4°C overnight. The homogenate was then mixed and diluted with three volumes of water (Super Q). The elongated spermatid nuclei resistant to homogenization were counted by using a hemacytometer. Because the average duration of the spermatid stage is 6.1 d, daily sperm production was calculated by dividing the spermatid number by 6.1 *(19).* The epididymal transit time of sperm was calculated by dividing the sperm number in epididymis by the daily sperm production *(19).*

Chemical Analysis

In experiment 2, urinary testosterone was measured by using an enzyme-linked immunoassay kit (Testosterone EIA Kit, Cayman Chemical, Ann Arbor, MI). Because urinary testosterone is a mixture of free and conjugated testosterone, urine samples were hydrolyzed with 0.5 *N* HCl in a boiling water bath for 15 min prior to analysis to obtain total testosterone.

Statistics

Data were analyzed by two-way ANOVA found in SYSTAT Version 5.0 (Systat, Inc., Evanston, IL). A p-value ≤0.05 was considered statistically significant.

RESULTS

In experiment 1, four rats in the nickel-deficient, L-NAME-supplemented group and two rats in the nickel-adequate, L-NAME-supplemented group died before the experiment was terminated. Thus, the number of rats available for analysis in these two groups were four and six, respectively, instead of eight. The deaths resulting from the administration of 50 mg L-NAME/100 mL drinking water was unexpected because the administration of this amount or higher of L-NAME has been used by other investigators without any apparent excessive morbidity or deaths in rats apparently fed natural-ingredient diets *(20–24).* Moreover, we have successfully used this treatment in other experiments in which rats were fed a ground corn–casein diet. The morbidity and deaths in experiment 1 suggest that rats are more sensitive to L-NAME when the diet is high in purified ingredients (i.e., sucrose). The rats fed L-NAME that gave the data presented had a normal appearance and their elevated blood pressures were not excessively high. In experiment 2, two rats in the nickel-deficient, NaCl-supplemented group and one rat in the nickel-adequate, NaCl-supplemented group died before the experiment was terminated. Thus, the number of rats available for analysis in these two groups were six and seven, respectively, instead of eight.

Table 2 shows that NaCl supplementation significantly depressed (p=0.002) final body weight. Tables 3 and 4 show that the weight of testes was not affected by the dietary treatments. The weight of epididymides was significantly decreased by L-NAME. The weights of seminal vesicles and of prostate, which were determined only in experiment 2, were significantly decreased by nickel deprivation. Excessive dietary NaCl also significantly decreased the weight of the prostate. Urinary testosterone excretion in experiment 2 was not affected by dietary nickel or NaCl.

Table 5 shows that in experiment 1 dietary nickel deprivation significantly decreased sperm motility, sperm count, and sperm density. L-NAME also significantly decreased sperm count and sperm density. Table 6 also shows that dietary nickel deprivation significantly decreased sperm motility, sperm count, and sperm density in experiment 2. Because of the findings in experiment 1, additional measurements of daily sperm production and epididymal transit time of spermatozoa were determined in experiment 2; both of these variables were significantly decreased by dietary nickel deprivation. Excessive NaCl did not significantly affect sperm measurements. The lowest values for all sperm measurements

Table 2
Effect of Dietary Nickel, L-NAME, and NaCl on Final Weight

Experiment 1[a]				Experiment 2[b]			
Ni[c]	L-NAME[c]	n	weight, g	Ni[d]	NaCl[d]	n	weight, g
+	-	8	296 ± 33[e]	+	-	8	425 ± 45
+	+	6	289 ± 46	+	+	7	387 ± 26
-	-	8	318 ± 27	-	-	8	447 ± 41
-	+	4	282 ± 28	-	+	6	389 ±29
ANOVA							
	Ni		0.58		Ni		0.42
	L-NAME		0.14		NaCl		0.002
	Ni x L-NAME		0.23		Ni x NaCl		0.47

[a] Rats fed respective treatments for 8 wk.
[b] Rats fed respective treatments for 16 wk.
[c] Variables were supplemental dietary nickel at 0 or 1 mg/kg and 0 or 50 mg L-NAME/100 mL drinking water (last 3 wk of experiment).
[d] Variables were supplemental dietary nickel at 0 or 1 mg/kg and NaCl at 0 or 80 g/kg.
[e] Data are means ± SD.

Table 3
Effect of Nickel Deprivation and L-NAME on Weights of Internal Sex Organs (Experiment 1)

Ni[a]	L-NAME[a]	Testes,g	Testes wt/Body wt[b]	Epididymides, g	Epididymis wt/Body wt[b]
+	-	2.89±0.24[c]	0.985±0.101	0.73±0.06	0.249±0.031
+	+	2.99±0.48	1.040±0.127	0.68±0.06	0.239±0.030
-	-	2.99±0.23	0.942±0.078	0.74±0.03	0.233±0.015
-	+	2.74±0.37	0.976±0.145	0.67±0.09	0.238±0.032
ANOVA					
Ni		0.57	0.24	0.93	0.45
L-NAME		0.60	0.33	0.03	0.83
Ni x L-NAME		0.21	0.82	0.68	0.48

[a] Variables were supplemental dietary nickel at 0 or 1 mg/kg and 0 or 50 mg L-NAME/100 mL drinking water (last 3 wk of experiment).
[b] ×100.
[c] Data are means ± SD.

Table 4
Effect of Nickel Deprivation and Excessive Dietary Sodium Chloride on Urinary Testosterone and Weights of Internal Sex Organs (Experiment 2)

		Testosterone,			Seminal Vesicle		
Ni[a]	NaCl[a]	Urinary, pmol/h	Testes, g	Epidiymides, g	Left, mg	Right, mg	Prostate,mg
+	-	9.68±3.31[b]	3.41±0.24	1.17±0.09	356±65	348±61	1036±303
+	+	8.91±1.90	3.30±0.44	1.05±0.18	377±117	380±98	697±117
-	-	8.52±2.08	3.35±0.23	1.04±0.09	279±54	284±46	765±210
-	+	8.93±1.77	3.42±0.56	1.04±0.22	284±76	286±75	590±259
ANOVA							
Ni		0.50	0.22	0.22	0.01	0.008	0.04
NaCl		0.83	0.32	0.32	0.68	0.55	0.006
Ni x NaCl		0.48	0.30	0.30	0.80	0.60	0.34

[a] Variables were supplemental dietary nickel at 0 or 1 mg/kg and NaCl at 0 or 80 g/kg.
[b] Data are means ± SD.

Table 5
Effect of Nickel Deprivation and L-NAME on Sperm Motility, Count, and Density in Right Epididymis (Experiment 1)

Ni[a]	L-NAME[a]	Sperm		
		motile %	Count, 10^6	Density, 10^6/g
+	-	6.8 ± 2.7[2]	153 ±15	425 ± 45
+	+	-[c]	113 ± 10	337 ± 48
-	-	2.0 ± 1.0	139 ± 2	337 ± 54
-	+	-[c]	78 ± 17	245 ± 26
ANOVA				
Ni		0.0003[d]	0.003	0.003
L-NAME		-	<0.0001	<0.0001
Ni x L-NAME		-	0.19	0.29

[a] Variables were supplemental dietary nickel at 0 or 1 mg/kg and 0 or 50 mg L-NAME/100 mL drinking water (last 3 wk of experiment).
[b] Data are means ± SD.
[c] Not measured.
[d] Determined by Student's *t*-test.

Table 6
Effect of Nickel Deprivation and Excessive Dietary Sodium Chloride on Sperm Motility, Count, and Density in Right Epididymus, and Sperm Production Rate and Epididymal Transit Time of Right Testis (Experiment 2)

Ni[a]	NaCl[a]	Sperm					Epididymal transit time, day
		saline motility,%	medium 199 motility, %	count, 10^6	density 10^6/g	production rate, 10^6/day	
+	-	4.73 ± 1.95[b]	7.64 ± 3.07	232 ± 54	395 ± 90	27.8 ± 1.8	8.28 ± 1.66
+	+	4.23 ± 2.26	8.42 ± 2.93	193 ± 50	360 ± 56	27.7 ± 4.3	7.47 ± 1.83
-	-	2.06 ± 1.02	3.59 ± 0.68	161 ± 37	301 ± 45	25.3 ± 2.8	6.20 ± 1.46
-	+	1.16 ± 0.76	2.80 ± 1.41	141 ± 30	256 ± 25	22.8 ± 5.4	6.77 ± 1.30
ANOVA							
Ni		0.001	0.001	0.003	0.001	0.01	0.03
NaCl		0.26	0.99	0.13	0.11	0.36	0.84
Ni x NaCl		0.75	0.37	0.41	0.96	0.40	0.26

[a] Variables were supplemental dietary nickel at 0 or 1 mg/kg and NaCl at 0 or 80 g/kg.
[b] Data are means ± SD.

except epididymal transit time, however, were found in the nickel-deficient rats fed excessive NaCl. In experiment 2, it was found that sperm motility was generally higher in Medium 199 than in saline.

DISCUSSION

Both deficient and excessive intakes of nickel affect reproductive performance in experimental animals. The findings with nickel deprivation are described in the Introduction. Excessive nickel intake by male rats results in increased testicular nickel, shrinkage of seminiferous tubules, decreased spermatids, and decreased fertility *(25)*. The relative order of bioaccumulation of subchronic oral nickel is kidney>testes> lung=brain according to Obone et al. *(26)*. These effects of various oral nickel intakes indicate that the blood–testis barrier *(27)* is permeable to nickel, which is necessary if nickel is needed for a function in the testis. This need is confirmed by the finding in the present study that nickel deprivation decreases the sperm production rate in testis, sperm counts in the epididymis, epididymal transit time of spermatozoa, and sperm motility collected from the entire epididymis. Because both excessive and deficient dietary nickel diminish physiological characteristics of spermatozoa, which most likely could result in impaired reproductive performance, there must be an optimum intake of nickel for reproduction.

Because dietary nickel did not affect the urinary excretion of testosterone, the effects of nickel deprivation on sperm characteristics and on

seminal vesicle and prostate size probably did not occur through changes in endocrine function. Instead, the changes most likely occurred through another means.

One possible mechanism through which nickel could affect sperm motility is by altering CNG cation channel function. This suggestion seems plausible because of the findings showing that nickel can potentiate the action of some of these channels *(3–5,28)*, and studies of several animal species indicate that spermatozoa motility involves cGMP and CNG channels. Hamster spermatozoa movement is triggered by cGMP *(29)*. Ovarian follicular fluid and atrial natriuretic peptide (ANP) attract human spermatozoa through cGMP production that triggers the movement of flagella *(30)*. Weisner et al. *(12)* found that mature bovine spermatozoa possess CNG α-subunits along the entire flagellum, and CNG short β-subunits on the principal piece of the flagellum; the CNG channels served as a Ca^{2+} entry point that responded more sensitively to cGMP than to cAMP.

The finding that nickel affected epididymal sperm density and transit time also might be related to cGMP action. Spermatozoa are produced in seminiferous tubules in testes and carried into epididymides via the rete testis and ductuli efferentes, which are derived from the tubules of the embryonic mesonephric kidney and are homologous to the renal proximal tubules. In the kidney, CNG channels occur in the cortical collecting duct and inner medullary collecting ducts. In the inner medullary collecting ducts, the CNG channel is inhibited by cGMP, which is formed when ANP binds to the guanylate cyclase-A receptor. The closure of the CNG cation channel decreases sodium reabsorption and exerts natriuresis *(31,32)*. Because of the ontogenic similarity to the renal tubules, cGMP may have physiological roles in the ductal system of male reproductive organs *(33)*. The epididymis ducts in rats isosmotically absorb more than 95% of the fluid leaving the testis. The fluid reabsorption determines the epididymal sperm density as well as the transit time that affects sperm maturation *(33)*. Transit time also may be affected by changes in the smooth muscle peristalsis of the epididymal ductal systems, which is affected by the ANP–cGMP and NO–cGMP signal transduction systems.

Other evidence indicating that CNG channels are crucial to the male reproductive system, including those activated by cGMP and that might be affected by nickel is the presence of substances involved in cGMP production and metabolism throughout the system. Soluble guanylate cyclase, nitric oxide synthase, and guanylate cyclase B in addition to cGMP are found in the lamina propria of the semiferous tubule, and Sertoli cells, Leydig cells, and blood vessels in human testes *(34,35)*. Cyclic nucleotide phosphodiesterases that hydolyze cGMP and cAMP have been found in somatic and germ cells of mouse and rat seminiferous tubules in the testis *(36)*.

In summary, dietary nickel deprivation diminished sperm density and motility in the epididymis and sperm production rate by the testes. Variables affected by nickel were affected similarly by L-NAME treatment. Although the only sex variable significantly affected by high dietary NaCl

similarly to nickel deprivation was a decreased prostate weight, the combined high dietary NaCl and nickel deprivation seemed to be the most detrimental to sperm motility, count, density, and production rate. Thus, the findings in the present study combined with those of other studies showing that nickel can potentiate or desensitize cGMP channels support the hypothesis that nickel has an essential function that affects CNG channels. The findings also indicate that a low nickel status can affect reproductive performance in higher animals.

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REFERENCES

1. F. H. Nielsen, D. R. Myron, S. H. Givand, et al., Nickel deficiency in rats, *J. Nutr.* **105,** 1620–1630 (1975).
2. M. Anke, B. Groppel, U. Krause, et al., Further data on the biological essentiality of nickel, in *Trace Elements in Man and Animals 6,* L. S. Hurley, C. L. Keen, B. Lonnerdal, et al., eds., Plenum, New York, pp. 467–469 (1988).
3. M. Ildefonse and N. Bennett, Single-channel study of the cGMP-dependent conductance of retinal rods from incorporation of native vesicles into planar bilayers, *J. Membr. Biol.* **123,** 133–147 (1991).
4. M. Ildefonse, S. Crouzy, and N. Bennett, Gating of retinal rod cation channel by different nucleotides: comparative study of unitary currents, *J. Membr. Biol.* **130,** 91–104 (1992).
5. J. W. Karpen, R. L. Brown, L. Stryer, et al., Interaction between divalent cations and the gating machinery of cyclic GMP-activated channels in salamander retinal rods, *J. Gen. Physiol.* **101,** 1–25 (1993).
6. P. A. Kingston, F. Zufall, and C. J. Barnstable, Rat hippocampal neurons express genes for both rod retinal and olfactory cyclic nucleotide-gated channels: novel targets for cAMP/cGMP function, *Proc. Natl. Acad. Sci. USA* **93,** 10,440–10,445 (1996).
7. J. Bradley, Y. Zhang, R. Bakin, et al., Functional expression of the heteromeric "olfactory" cyclic nucleotide-gated channel in the hippocampus: a potential effector of synaptic plasticity in brain neurons, *J. Neurosci.* **17,** 1993–2005 (1997).
8. I. Ahmad, C. Korbmacher, A. S. Segal, et al., Mouse cortical collecting duct cells show nonselective cation channel activity and express a gene related to the cGMP-gated rod photoreceptor channel, *Proc. Natl. Acad. Sci. USA* **89,** 10,262–10,266 (1999).
9. M. Biel, X. Zong, M. Distler, et al., Another member of the cyclic nucleotide-gated channel family, expressed in testis, kidney, and heart, *Proc. Natl. Acad. Sci. USA* **91,** 3505–3509 (1994).

10. M. Biel, W. Altenhofen, R. Hullin, et al., Primary structure and functional expression of a cyclic nucleotide-gated channel from rabbit aorta, *FEBS Lett.* **329,** 134–138 (1993).
11. I. Weyand, M. Godde, S. Frings, et al., Cloning and functional expression of a cyclic-nucleotide-gated channel from mammalian sperm, *Nature* **368,** 859–863 (1994).
12. B. Wiesner, J. Weiner, R. Middendorff, et al., Cyclic nucleotide-gated channels on the flagellum control Ca^{2+} entry into sperm, *J. Cell Biol.* **142,** 473–484 (1998).
13. D. Ritter, A. D. Dean, S. L. Gluck, et al., Natriuretic peptide receptors A and B have different cellular distribution in rat kidney, *Kidney Int.* **48,** 5758–5766 (1995).
14. Y. Terada, K. Tomita, H. Nonoguchi, et al., PCR localization of C-type natriuretic peptide and B-type receptor mRNAs in rat nephron segments, *Am. J. Physiol.* **267,** F215–F222 (1994).
15. J. Navarro, A. Sanchez, J. Saiz, et al., Hormonal, renal, and metabolic alterations during hypertension induced by chronic inhibition of NO in rats, *Am. J. Physiol.* **267,** R1516–R1521 (1994).
16. P. Burguera, A. Sanchez de Briceno, C. E. Rondon, et al., Determination of nickel in saliva by electrothermal atomic absorption spectrometry using various chemical modifiers with Zeeman effect background correction, *J. Trace Elements Med. Biol.* **12,** 115–120 (1998).
17. R. L. Dahlquist and J. W. Knoll, Inductively coupled plasma–atomic emission spectrometry: analysis of biological materials and soils in major, trace and ultra-trace elements, *Appl. Spectrosc.* **32,** 1–29 (1978).
18. P. G. Reeves and K. L. Rossow, Zinc deficiency affects the activity and protein concentration of angiotensin-converting enzyme in rat testes, *Proc. Soc. Exp. Biol. Med.* **203,** 336–342 (1993).
19. G. W. Robb, R. P. Amann, and G. J. Killian, Daily sperm production and epididymal sperm reserves of pubertal and adult rats, *J. Reprod. Fertil.* **54,** 103–107 (1978).
20. A. Fernandez-Rivas, J. Garcia-Estan, and F. Vargas, Effects of chronic increased salt intake on nitric oxide synthesis inhibition-induced hypertension, *J. Hypertens.* **13,** 123–128 (1995).
21. A. Swislocki, T. Eason, and C. A. Kaysen, Oral administration of the nitric oxide biosynthesis inhititor, *N*-nitro-L-arginine methyl ester (L-NAME), causes hypertension, but not glucose intolerance or insulin resistance, in rats, *Am. J. Hypertens.* **8,** 1009–1014 (1995).
22. H. Tsukahara, T. Imura, S. Tsuchida, et al., Renal functional measurements in young rats with chronic inhibition of nitric oxide synthase, *Acta Paediatr. Jpn.* **38,** 614–618 (1996).
23. S. L. Lubarsky, R. A. Ahokas, S. A. Friedman, et al., The effect of chronic nitric oxide synthesis inhibition on blood pressure and angiotension II responsiveness in the pregnant rat, *Am. J. Obstet. Gynecol.* **176,** 1069–1076 (1997).
24. N. Akuzawa, T. Nakamura, T. Kurashina, et al., Antihypertensive agents prevent nephrosclerosis and left ventricular hypertrophy induced in rats by prolonged inhibition of nitric oxide synthesis, *Am. J. Hypertens.* **11,** 697–707 (1998).
25. R. Kakela, A. Kakela, and H. Hyvarinen, Effects of nickel chloride on reproduction of the rat and possible antagonistic role of selenium, *Comp. Biochem. Physiol. C* **123,** 27–37 (1999).
26. E. Obone, S. K. Chakrabarti, C. Bai, et al., Toxicity and bioaccumulation of nickel sulfate in Sprague–Dawley rats following 13 weeks of subchronic exposure, *J. Toxicol. Environ. Health* **57A,** 379–401 (1999).
27. L. M. King, W. A. Banks, and W. J. George, Differential zinc transport into testis and brain of cadmium-sensitive and -resistant murine strains, *J. Androl.* **21,** 656–663 (2000).
28. S. E. Gordon and W. N. Zagotta, Subunit interactions in coordination of Ni^{2+} in cyclic nucleotide-gated channels, *Proc. Natl. Acad. Sci. USA* **92,** 10,222–10,226 (1995).
29. B. Morton, J. Harrigan-Lum, L. Albagli, et al., The activation of motility in quiescent hamster sperm from the epididymis by calcium and cyclic nucleotides, *Biochem. Biophys. Res. Commun.* **56,** 372–379 (1974).

30. R. A. Anderson, Jr., K. A. Feathergill, R. G. Rawlins, et al., Atrial natriuretic peptide: a chemoattractant of human spermatozoa by a guanylate cyclase-dependent pathway, *Mol. Reprod. Dev.* **40,** 371–378 (1995).
31. D. E. McCoy, S. E. Guggino, and B. A. Stanton, The renal cGMP-gated cation channel: its molecular structure and physiological role, *Kidney Int.* **48,** 1125–1133 (1995).
32. D. H. Vandorpe, F. Ciampolillo, R. B. Green, et al., Cyclic nucleotide-gated cation channels mediate sodium absorption by IMCD (mIMCD-K2) cells, *Am. J. Physiol.* **272,** C901–C910 (1997).
33. J. C. Hansen and R. C. Jones, In vivo microperfusion of the ductuli efferentes testis of the rat: flow dependence of fluid reabsorption, *Exp. Physiol.* **81,** 633–644 (1996).
34. R. Middendorff, M. S. Davidoff, S. Behrends, et al., Multiple roles of the messenger molecule cGMP in testicular function, *Andrologia* **32,** 55–59 (2000).
35. R. Middendorff, D. Muller, H. J. Paust, et al., New aspects of Leydig cell function, *Adv. Exp. Med. Biol.* **424,** 125–138 (1997).
36. P. Rossi, R. Pezzotti, M. Conti, et al., Cyclic nucleotide phosphodiesterases in somatic and germ cells of mouse seminiferous tubules, *J. Reprod. Fertil.* **74,** 317–322 (1985).